IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number	:	10/575,236	Confirmation No.: 6957
Applicant	:	Alain GOUGEON, et al.	
Filed	:	November 16, 2006	
Title	:	**USE OF SOMATOSTATIN OR ONE OF ITS ANALOGUES FOR PREPARING A MEDICAMENT INTENDED TO REGULATE THE OVARIAN FOLLICULAR RESERVE IN NON-MENOPAUSAL WOMEN**	
TC/Art Unit	:	1654	
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SUBMISSION OF ENGLISH TRANSLATION OF PRIORITY DOCUMENT AND CERTIFICATE OF THE TRANSLATOR

Sir:

Applicants respectfully submit the English translation of the certified copy of French Patent Application No. 03292505.9, filed October 10, 2003, along with a certificate of the translator in connection with the above-identified patent application. Applicants claim priority benefits under 35 U.S.C. § 119(e) from this French Application in the above identified Application.

No fee is believed due as a result of this submission. However, if a fee is due upon the filing of this priority document, please charge such fee to the undersigned's Deposit Account No. 50-0206.

Respectfully submitted,

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Dated: October 1, 2009

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English Translation

I, Anne Kristine Kjelsen, domiciled at Adrigole (Beara), Co. Cork, Republic of Ireland, translator, do hereby solemnly and sincerely declare:

1. that I am acquainted with the French language;

2. that I am acquainted with the English language;

3. that I have translated resp. duly reviewed the translation of the document indicated hereinafter;

4. and that to the best of my knowledge and belief the following is a true and correct English translation thereof.

Adrigole, August 13, 2009

Anne Kristine Kjelsen

PATENT APPLICATION

Country	France
Application N°	EP03292505.9
Filing Date	10.10.2003 (10 October 2003)
Applicant	Institut National de la Santé et de la Recherche médicale (INSERM), 101, rue de Tolbiac, F-75013 Paris, France Société de Conseils de Recherches et d'Applications Scientifiques (SCRAS), 42 rue du Docteur Blanche, F-75781 Paris Cédex 16, France
Title in French	Utilisation de la somatostine ou d'un de ses analogues pour préparer un médicament destine à réguler la réserve folliculaire ovarienne chez la femme non ménopausée
Title in English	Use of somatostatin or one of its analogues for preparing a medicament intended to regulate the ovarian follicular reserve in non-menopausal women

Use of somatostatin or one of its analogues for preparing a medicament intended to regulate the ovarian follicular reserve in non-menopausal women

The invention relates to the use of somatostatin or one of its agonist analogues for preparing a medicament intended to regulate the ovarian follicular reserve in non-menopausal women, or the use of a somatostatin antagonist analogue for preparing a medicament intended to accelerate the start of growth of the quiescent follicles in non-menopausal women.

In women, as in all mammals, fertility is dependent on the presence in the ovaries of female gametes called "oocytes". In humans, the oocyte capital is constituted once and for all at birth; the number of oocytes is then comprised between 500,000 and 1 million per ovary. These oocytes are surrounded by a few granulosa cells; this functional group is called an ovarian follicle (Gougeon, A., *Endocrine Reviews* (1996), **17**, 121-155). At birth, but also throughout life until menopause, the majority of the ovarian follicles are in a dormant state.

From its constitution, the oocyte capital progressively diminishes: thus, there are approximately 200,000 follicles per ovary at puberty, approximately 80,000 at 20 years of age, approximately 30,000 at 30 years of age, approximately 10,000 at 40 years of age, the capital being practically depleted at around 50 years of age (cf. Gougeon, A. and Lefèvre, B., «Folliculogénèse et maturation ovocytaire» in *Médecine de la reproduction*, 3rd edition, Ed. Flammarion, p. 49). The depletion of the oocyte capital corresponds clinically to menopause. The dormant follicles present in the ovary at a given time constitute the "ovarian reserve".

Two mechanisms are involved in the progressive depletion of the ovarian reserve. Approximately 80% of the follicles disappear at the start of apoptosis, while the remaining 20% start to grow. The latter then begin a long process of development (approximately 6 months) in which a minority of them (approximately 400 over a lifetime) will arrive at the stage of preovulatory follicles containing a mature oocyte capable of being fertilized (Gougeon, A., *Endocrine Reviews* (1996), **17**, 121-155). The majority of the growing follicles disappear through apoptosis leading to their involution; apoptosis strikes them at any stage of their development.

The change from the quiescent follicle stage to the growing follicle stage is a phenomenon which is continuous but of variable intensity. In particular, it accelerates in the 10 to 15 years preceding menopause, from approximately 38 years of age.

The factors stimulating the first stages of growth (starting from the large primary follicle) are relatively well known. They include gonadotropins (LH and FSH) but particularly growth factors and steroids such as androgens. However, the mechanisms controlling the initiation of follicle growth are not well known. It is well established that this stage of folliculogenesis is not dependent on gonadotropins (LH and FSH) (see for example Bullun, S. and Adashi, E., *Williams Textbook of Endocrinology*, Tenth Edition (2003), 587-664). A hormone known as AMH (Anti-Mullerian hormone) could be involved in maintaining the quiescence of the follicles while a peptide known as Kit-Ligand (also called SCF) could be involved in activating the growth of dormant follicles. In addition, a growth factor known as GDF-9 seems to be important for maintaining the growth once it is triggered.

Somatostatin (SST) is a cyclic peptide present in two forms in the organism, one form containing 14 amino acids and one form containing 28 amino acids. The biological activity of these two forms of SST is similar. The SST-14 form is the predominant form in the central nervous system. It inhibits the secretion of the growth hormone by the somatotrope cells of the anterior pituitary. The SST-28 form is preferably expressed in the stomach and the pancreas. The biological activity of SST is induced by means of a series of membrane receptors coupled with a protein G, 5 sub-types of which have been characterized, namely the sub-types SSTR1 to SSTR5 (Reubi, J.C., *Cancer Res.*, **47**, 551-558; Resine, T., et al., *Endocr. Review*, **16**, 427 – 442; Lamberts, SW. et al., *Endocr. Review*, **12**, 450-482).

The presence of SST in the ovary has been demonstrated in several species including pigs (Mori, T. et al., *Acta Endocrinol. (Copenh.)* (1984), **106**(2), 254-259), rats (McNeill, D.L. et al., *Am. J. Anat.* (1987), **179**(3), 269-76) and in women (Holst et al., *Hum. Reprod.* (1994), **9**(8), 1448-1451). SST receptors have been identified in the ovary of the rat (Lidor, A. et al., *Gynecol. Endocrinol.* (1998), **12**(2), 97-101) as well as in the human ovary in particular the sub-types 1, 2A and 5 (Strauss et al., *Hum. Reprod.* (2003), **18**, Suppl. 1, P-495).

The contribution of SST in ovarian physiology has been studied by several authors. In rats, the *in vivo* administration of SST seems to reduce the number of pituitary cells producing LH and FSH as well as the number of preovulatory follicles in the ovary (Nestorovic et al., *Histochem. J.* (2001), **33**(11-12), 695-702). *In vitro*, SST inhibits aromatase and the production of progesterone, stimulated by FSH, in a model of granulosa cells (Andreani, C.L. et al., *Hum. Reprod.* (1995), **10**(8), 1968-1973). In pigs, SST inhibits the increase in cAMP induced by LH and forskolin in the granulosa cells (Rajkumar, K. et al., *J. Endocrinol.* (1992), **134**(2), 297-306), and seems to inhibit the

nuclear maturation of the preovulatory oocyte (Mori, T. et al., *Acta Endocrinol. (Copenh.)* (1985), **110**(3), 408-412). In women, *in vitro* studies on granulosa cells from preovulatory follicles suggest a direct role of SST in inhibiting the synthesis of IGF-BP1 and of progesterone (Holst, N. et al., *Fertil. Steril.* (1997), **68**(3), 478-482). In women, *in vivo*, SST is capable of reducing the secretion of LH by the pituitary, of reducing the production of androgens and the IGF-1 serum levels. By contrast, SST increases the serum levels of IGF-BP3 (Fulghesu, A.M. et al., *Fertil. Steril.* (1995), **64**(4), 703-708; Piaditis, G.P. et al., *Clin. Endocrinol. (Oxf.)* (1996), **45**(5), 595-604). SST was co-administered with FSH during treatment to induce ovulation in patients who are infertile as a result of a polycystic ovary syndrome. The capacity of SST to reduce the LH serum levels, and to reduce the serum levels of growth hormone and of IGF-I, has been confirmed. This endocrine effect is not however accompanied by a significant impact on the follicle growth induced by the administration of FSH (Lidor, A. et al., *Gynecol. Endocrinol.* (1998), **12**(2), 97-101; van der Meer, M. et al., *Hum. Reprod.* (1998), **13**(6), 1465-1469). In summary, until now a marginal effect of SST on the pituitary secretion of LH and on the production of progesterone by the granulosa cells of preovulatory follicles has been reported.

The applicants have now discovered, in a surprising manner, that SST and its analogues have the capacity to inhibit the transition of the follicles from a quiescent stage to a growth stage and that this faculty allows new therapeutic uses for these compounds.

The benefit of this discovery resides primarily in the possibility of using SST or an SST agonist analogue for preparing a medicament intended to diminish or even inhibit the start of growth of follicles in the quiescent stage. Secondly, it is also possible to use an antagonist analogue of this peptide in order to prepare a medicament intended to accelerate the start of growth of the quiescent follicles.

There exists a set of clinical situations for which it would be desirable from a medical perspective for the patient to slow the use of the ovarian reserve in order to delay the depletion of the latter and therefore to preserve the ovarian function and fertility. These situations are typically, and non-exclusively, patients at risk of early menopause. It is well known that certain patients have a premature depletion of their follicular capital. Menopause then occurs before 40 years of age and sometimes even before thirty years of age. It is often possible to predict this early menopause on the basis of family antecedents, or genetic anomalies such as Turner syndrome (complete or partial). In this situation, the administration of SST or of one of its agonist analogues is a preventive measure and aims to slow the start of growth of the quiescent follicles.

The same applies for patients having difficulty conceiving and for whom the chronological age or biological age of their ovaries corresponds to the period of acceleration of activation of the quiescent follicles: slowing this depletion of follicular capital should make it possible to increase the efficiency of the treatments and the chances of becoming pregnant.

Another clinical situation that may benefit from a treatment by SST or by one of its agonist analogues is the graft (preferably an autograft) of an ovary or of ovary fragments. In this context, the resumption of ovarian function is often temporary and is accompanied by a premature depletion of the number of primordial follicles (Baird, D.T. et al., *Endocrinology* (1999), **140**, 462-471). It has in fact been demonstrated that, during the transplantation, the granulosa cells of the growing follicles are more inclined to start an apoptosis phenomenon than those of the primordial follicles (Liu, J. et al., *Hum. Reprod.* (2002), **17**, 605-611). Moreover, the removal of ovarian tissue and its fragmentation causes the primordial follicles to move rapidly and en masse toward a stage of late primary follicles (cf. Wandji S-A, et al., *Hum. Reprod.* (1997), **12**, 1993-2001; see also the control group of the example of the present application).

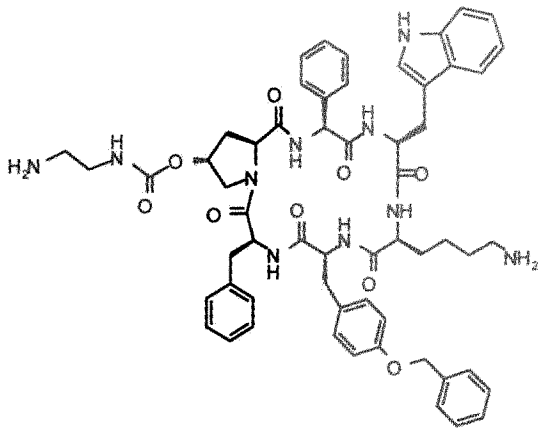
In addition to the pathologies mentioned above, a systematic slowing of the depletion of the ovarian reserve could be envisaged in women not suffering from any ovarian dysfunction. In industrialized countries, the continuous extension of life expectancy (currently approximately 83 years in France) is accompanied by an extension of the post-menopausal period and of the problems associated with it: heart diseases, osteoporosis, cutaneous aging, etc. Doubts are raised as to the long-term safety of hormonal substitute treatments for menopause. An attractive alternative would consequently be delaying the age at which menopause occurs. This would thus reduce the post-menopausal period and the associated risks. This delay would not however mean that fertility could be maintained up to 60 plus years of age. Numerous works suggest that ovarian function is maintained as long as a minimum number of follicles of the reserve is maintained, and that despite a "normal" ovarian function (steroid levels barely affected), the chances of pregnancy are extremely low.

In situations where it is sought to slow the use of the ovarian reserve, according to the invention natural somatostatin (SST14 or SST28), or, preferably, a somatostatin agonist analogue (natural or synthetic), will be used. The somatostatin agonist analogue can be a cyclic or non-cyclic polypeptide, a fusion or recombination protein, a non-peptidic chemical entity (i.e. peptidomimetic) or also an "*SS-like*" peptide such as corticostatin. The agonist analogues to be used must have high affinity for the SST receptor and induce a functional activity thereof such as the inhibition of the secretion of growth

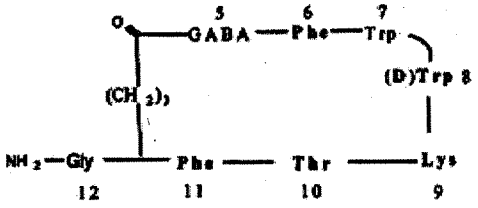
hormone by pituitary somatotrope cells and/or the inhibition of the in vitro proliferation of pituitary adenoma cells. Preferably, the somatostatin agonist analogue has high affinity for all types of SST receptors or a greater affinity for at least one of the sub-types 1, 2, 3, 4 and 5.

Agonist analogues of somatostatin have been described in particular in the patent application PCT WO 01/00676 or WO 98/08528 or also in the patents US 6,387,932, US 6,268,342, US 6,057,338, US 6,025,372.

Among the somatostatin agonist analogues which can be used according to the invention, one can mention more particularly lanreotide, octreotide, vapreotide, SOM 230 (see structure below), MK-678 (peptide of structure cyclo(N-Me-Ala-Tyr-D-Trp-Lys-Val-Phe)), BIM-23190 (peptide of structure N-hydroxyethylpiperazinyl-acetyl-D-Phe-cyclo[Cys-Tyr-D-Trp-Lys-Abu-Cys]-Thr-NH_2), BIM-23197 (peptide of structure Hepes-D-Phe-cyclo[Cys-Tyr-D-Trp-Lys-Abu-Cys]-Thr-NH_2 in which Abu represents aminobutyric acid), BIM-23268 (peptide of structure cyclo[Cys-Phe-Phe-D-Trp-Lys-Thr-Phe-Cys]-NH_2), PTR-3173 (see structure below), TT-232 (of structure D-Phe-cyclo[Cys-D-Trp-Lys-Cys]-Thr-NH_2), and their pharmaceutically acceptable salts. The use of lanreotide, octreotide or one of their pharmaceutically acceptable salts, and more particularly lanreotide or one of its pharmaceutically acceptable salts is quite particularly preferred.



SOM 230



PTR 3173

According to a preferred variant of the invention, the patients for whom the medicament based on somatostatin or a somatostatin agonist analogue mentioned above is intended are women having an early menopause risk factor, and in particular women with a family history of early menopause. According to a particular variant of the invention, the patients for whom the medicament based on somatostatin or somatostatin agonist analogue mentioned above is intended are women who have an X chromosome micro-deletion or a partial Turner syndrome.

The second benefit of the discovery mentioned above resides in the possibility of preparing a medicament based on a somatostatin antagonist analogue in order to accelerate the start of growth of the quiescent follicles. In fact, one couple in six of those who wish to achieve a pregnancy has difficulty conceiving. Although there are many causes, two types of treatment have emerged and are commonly used in human medicine for the treatment of sterility. These treatments, also called "Medically Assisted Procreation" (MAP), consist firstly in inducing the simultaneous growth of several preovulatory follicles. This makes it possible to obtain several mature oocytes, and therefore several embryos, and thus to increase the chances of conception. This is achieved by the administration of one or more medicaments stimulating the pituitary secretion of gonadotropins (FSH and LH), such as an anti-estrogen (for example clomiphene citrate or tamoxifen) or an aromatase inhibitor (for example letrozole, anastrazole or exemestane). The simultaneous growth of several preovulatory follicles can also be induced by the administration of a preparation of human FSH (extractive or recombinant) combined or not with LH. When the follicles have reached a preovulatory size, depending on the cause of sterility, two treatment options exist. The first is to carry out an intrauterine insemination (IUI) and the second is to remove the oocytes from the ovary by aspiration of the follicles (between 5 and 15 oocytes) and to carry out an insemination in the laboratory (*in vitro*), either by simple co-incubation of the oocytes with the partner's sperm (IVF) or by microinjection of sperm directly into the oocyte (ICSI). It is essential to obtain several mature oocytes in order to optimize the success rates (pregnancy rates) achieved with these treatments; however in certain women,

despite appropriate ovarian stimulation, the number of oocytes obtained is low or even equal to one. This difficulty in responding to the stimulating treatment is a result of the limited number of growing follicles present in the ovaries of these patients. It is therefore of considerable therapeutic benefit to be able to activate follicles of the ovarian reserve and make them enter the growth phase.

Another object of the present invention is the use of a somatostatin antagonist analogue for preparing a medicament intended to accelerate the start of growth of the quiescent follicles in non-menopausal women.

The administration of such a medicament over a period of 1 to 12 months in women leads to an increase in the number of follicles in the growth phase and which are therefore able to be stimulated with the standard treatments in order to reach the stage of preovulatory follicles.

The somatostatin antagonist analogue can be a cyclic or non-cyclic polypeptide, a fusion or recombination protein, a non-peptidic chemical entity (i.e. a peptidomimetic) or also a *"SS-like"* peptide such as corticostatin. The antagonist analogues to be used must have a high affinity for the SST receptor and inhibit the functional activity of SST14 or SST28 such as the inhibition of the secretion of growth hormone by pituitary somatotrope cells and/or the inhibition of the in vitro proliferation of pituitary adenoma cells. Preferably, the somatostatin antagonist analogue has a high affinity for all types of SST receptors or a greater affinity for at least one of the sub-types 1, 2, 3, 4 and 5.

A somatostatin antagonist analogue that can be used for the preparation according to the invention can for example be a peptide of general formula

$$A^1\text{-cyclo}\{\text{D-Cys-}A^2\text{-D-Trp-}A^3\text{-}A^4\text{-Cys}\}\text{-}A^5\text{-}Y^1$$

(I)

in which:

A^1 is an optionally substituted aromatic α-amino acid;

A^2 is an optionally substituted aromatic α-amino acid;

A^3 is Dab, Dap, Lys or Orn;

A^4 is β-Hydroxyvaline, Ser, Hser, or Thr;

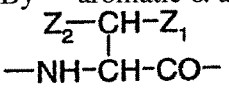
A^5 is an optionally substituted aromatic D- or L- α-amino acid; and

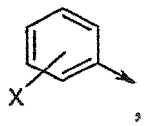
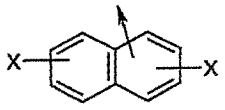
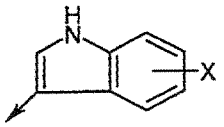
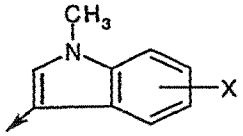
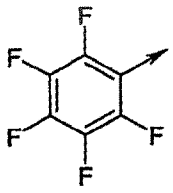
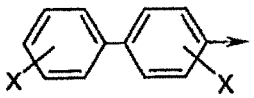
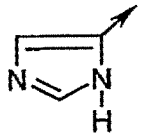
Y^1 is OH, NH_2 or NHR^1, R^1 being $(C_{1\text{-}6})$alkyl;

each optionally substituted aromatic α-amino acid being optionally substituted with one or more substituents independently chosen from the group comprising a halogen atom and the groups NO_2, OH, CN, (C_{1-6})alkyl, (C_{2-6})alkenyl, (C_{2-6})alkynyl, (C_{1-6})alkoxy, Bzl, O-Bzl and NR^9R^{10}, R^9 and R^{10} each being independently H, O, or (C_{1-6}) alkyl; and

each nitrogen atom with a peptide amide bond and the amino group of A^1 being optionally substituted with a methyl group, it being understood that there is at least one such methyl group in a peptide of general formula **(I)**;

or a pharmaceutically acceptable salt of a peptide of general formula **(I)**.

By "aromatic α-amino acid" is meant an amino acid residue of formula  , in which Z_1 is a radical containing an aromatic ring and Z_2 is a hydrogen atom or a radical containing an aromatic ring. Examples of such radicals containing an aromatic ring include, but are not limited to, a benzene or pyridine ring and the following structures with or without one or more X substituents on the aromatic ring (X being, independently each time that it occurs, a halogen atom, NO_2, CH_3, OCH_3, CF_3 or OH):

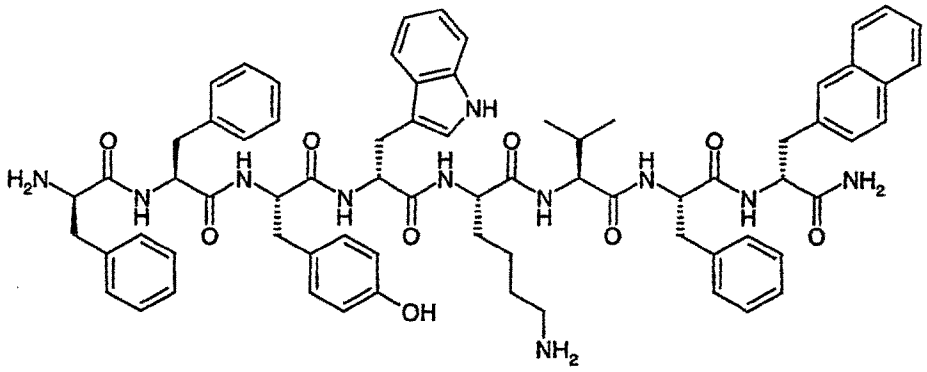
 ,  ,  ,  ,  ,  , and  . Other examples of an "aromatic α-amino acid" according to the invention are substituted His, such as MeHis, His (τ-Me) or His (π-Me).

Other somatostatin antagonist analogues have been described in particular in the patent applications PCT WO 98/08528, WO 98/08529, WO 98/24807, WO 98/44921, WO 98/44922, WO 98/45285 and WO 99/22735, or also in the patents US 6,387,932, US 6,262,229, US 6,063,796, US 6,057,338, US 6,025,372, US 5,925,618, US 5,846,934 and US 4,508,711.

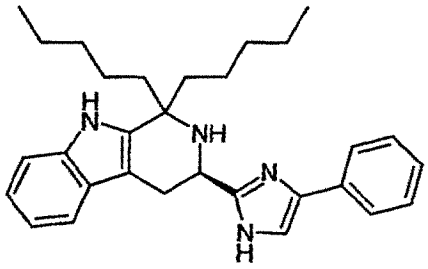
Among the somatostatin antagonist analogues which can be used according to the invention and their pharmaceutically acceptable salts, one can mention more particularly:

- ❖ the following peptides of general formula **(I)**:
  - Cpa-cyclo[D-Cys-Pal-D-Trp-N-Me-Lys-Thr-Cys]-D-Trp-NH_2;
  - Cpa-cyclo[D-Cys-Tyr-D-Trp- N-Me-Lys-Thr-Cys]-Nal-NH_2;
  - Cpa-cyclo[D-Cys-Pal-D-Trp- N-Me-Lys-Thr-Cys]-Nal-NH_2;
- ❖ the peptide known by the code name AC-178,335 (of structure acetyl-D-His-D-Phe-D-Ile-D-Arg-D-Trp-D-Phe-NH_2);
- ❖ the octapeptide known by the code name ODN-8 (see Fig. 1 of *Proc. Natl. Acad. Sci. USA* (2000), **97**(25), 13973–13978);
- ❖ the peptide known by the code name SB-710411 (of structure Cpa-cyclo[D-Cys-Pal-D-Trp-Lys-Val-Cys]-Cpa-amide);
- ❖ the peptide known by the code name BIM-23056 (of the structure represented below);
- ❖ the compound known by the code name BN-81674 (of the structure represented below);
- ❖ the compound known by the code name SRA-880 (of the structure represented below);

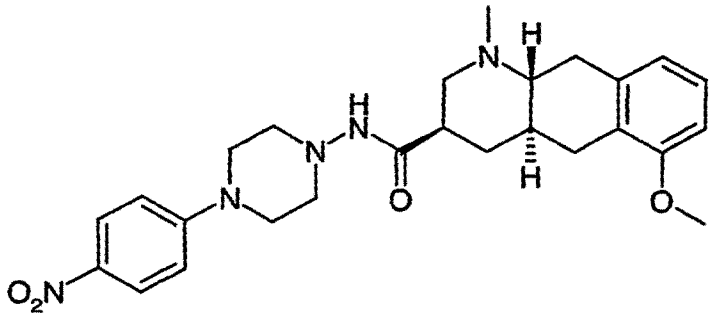
and their pharmaceutically acceptable salts.



BIM 23056



BN-81674



SRA-880

A somatostatin agonist analogue in the present application means a compound for which the effective dose DE_{50} determined in the test of the agonist effect described below is less than or equal to 1 μM for at least one of the somatostatin sub-receptors.

A somatostatin antagonist analogue in the present application means a compound for which the effective dose DE_{50} determined in the test of the antagonist effect described below is less than or equal to 1 μM for at least one of the somatostatin sub-receptors.

By pharmaceutically acceptable salt, one means notably in the present application addition salts with inorganic acids such as hydrochloride, hydrobromide, hydroiodide, sulphate, phosphate, diphosphate and nitrate or with organic acids such as acetate, maleate, fumarate, tartrate, succinate, citrate, lactate, methanesulphonate, p-toluenesulphonate, pamoate and stearate. Also included in the field of the present invention, when they can be used, are the salts formed from bases such as sodium or potassium hydroxide. For other examples of pharmaceutically acceptable salts, reference can be made to "Salt selection for basic drugs", *Int. J. Pharm.* (1986), **33**, 201-217.

According to the present invention, the pharmaceutical preparations containing somatostatin or one of its agonist or antagonist analogues applicable in this invention can be administered by parenteral route (subcutaneous, intramuscular, intraperitoneal, intravenous, or in an implant), by oral, vaginal, rectal, nasal, sublingual or transdermal route. The vaginal route is preferred because it allows effective concentrations of the active ingredient to be delivered to the ovary while minimizing systemic exposure. The somatostatin or the somatostatin analogue used is formulated with the necessary excipients known to a person skilled in the art, in order to allow an effective and reproducible administration for each administration route.

The dose of a product according to the present invention, intended for the treatment of the above-mentioned diseases or problems, varies according to the method of administration, the age and body weight of the subject to be treated as well as the latter's condition, and the final decision is made by the attending doctor or vet. Such a quantity determined by the attending doctor or vet is called here "therapeutically effective quantity".

The following typical situations for a use according to the invention could however be envisaged:

- A patient of approximately 20 to 25 years of age (for example) has a partial Turner syndrome through X chromosome micro-deletion. Her ovarian function is apparently normal with regular ovulatory cycles. Her FSH serum level is slightly higher during the luteal-follicular transition period (for example FSH = approximately 9.2 IU/liter). The ovarian ultrasound carried out by trans-vaginal route shows ovaries of normal volume with a slightly reduced number of antral follicles. Considering the high risk that she will have early menopause, the patient is treated with lanreotide acetate at a dose of 120 mg/months (Somatuline® Autogel® 120 mg, Beaufour Ipsen Pharma, France). The treatment is discontinued after several years when the patient wishes to conceive.

- A patient of approximately 35 to 40 years of age has had primary sterility for several years. Assessment of the couple produced a diagnosis of sterility of tubal origin, very probably resulting from a history of peritonitis. The menstrual cycles are ovulatory and the FSH serum level is slightly higher during the luteal-follicular transition period (for example FSH = approximately 11.4 IU/L). The ovarian ultrasound carried out by trans-vaginal route shows ovaries with a slightly reduced volume with a reduced number of antral follicles (approximately 3 per ovary). A diagnosis of reduction of the ovarian reserve is made. An *in vitro* fertilization treatment is recommended and the patient undergoes ovarian stimulation treatment

with daily injection of 225 units of recombinant FSH. On the 6th day of stimulation, an ovarian ultrasound shows a single growing follicle of 14 mm in the right ovary. The dose of FSH is doubled and the patient is seen again 2 days later. A single 18-mm follicle is observed, which confirms a reduction in the ovarian reserve. The treatment is discontinued. After return of a spontaneous cycle, a treatment by daily administration of a somatostatin antagonist analogue is initiated. During this treatment, the number of antral follicles present in each ovary is assessed by ultrasound at the start of each menstrual cycle. After 4 months of treatment, the number of antral follicles is on average approximately 6 per ovary and the serum FSH has been reduced. A stimulation by recombinant FSH is initiated, multiple follicular development is obtained, and a standard *in vitro* fertilization procedure is carried out.

Particular abbreviations and definitions used in the present application:

The abbreviations of the common amino acids are in accordance with the IUPAC-IUB recommendations. Moreover, the definitions for certain abbreviations used in the present application are as follows:

Abu	=	α-aminobutyric acid;
Aib	=	α-aminoisobutyric acid;
β-Ala	=	β-alanine;
Amp	=	4-aminophenylalanine;
Ava	=	5-aminovaleric acid;
Cha	=	cyclohexylalanine;
Cpa	=	3-(4-chlorophenyl)alanine;
Dab	=	2,4-diaminobutyric acid;
Dap	=	2,3-diaminopropionic acid;
Dip	=	3,3'-diphenylalanine;
GABA	=	γ-aminobutyric acid;
HSer	=	homoserine;

1-Nal	=	3-(1-naphthyl)alanine;
2-Nal	=	3-(2-naphthyl)alanine;
Nle	=	norleucine;
Nva	=	norvaline;
2-Pal	=	3-(2-pyridyl)alanine;
3-Pal	=	3-(3-pyridyl)alanine;
4-Pal	=	3-(4-pyridyl)alanine;
Tfm	=	trifluoromethyl;
TfmA	=	4-trifluoromethylphenyl-alanine;

Finally, Tyr(I) represents an iodized tyrosine residue (for example 3-1-Tyr, 5-I-Tyr, 3,5-I-Tyr) in which the iodine atom can be a radioactive isotope, for example I_{125}, I_{127} or I_{131}.

Moreover, the term "approximately" refers to an interval around the value considered. As used in the present application, "approximately X" means an interval of X less 10% of X to X plus 10% of X, and preferably an interval of X less 5% of X to X plus 5% of X.

Preparation of the peptides of general formula (I):

The peptides of general formula **(I)** mentioned above and their synthesis are described for instance in patent application PCT WO 02/072602.

Unless they are otherwise defined, all the technical and scientific terms used here have the same meaning as that usually understood by an ordinary specialist in the field to which this invention belongs. Similarly, all the publications, patent applications, all the patents and all other references mentioned here are incorporated by way of reference.

The following examples are given in order to illustrate the above procedures and must in no event be considered to be a limit to the scope of the invention.

EXAMPLE:

Ovaries of adult ewes are collected immediately after slaughter. The ovaries are placed in an organ transport medium without serum (X-vivo, Bio Whittaker, Walkersville, MD, USA) at 10°C and transported to the laboratory. Approximately 1 h after removal, the cortex is isolated from the medulla then fractionated into slices of 2 mm thickness (1 cm^2, average weight of 212 mg) after rinsing in new X-vivo. The cortex fragments are cultured in an oven under 5% oxygen for 10 days in well plates in the presence of DMEM. The medium is changed every 2 days.

In the control fragments (incubated in the absence of SST) the primordial follicles gradually progress to the state of follicles at the start of growth (see Figures 1 and 2). The addition of SST14 at concentrations varying between 10^{-9} M and 10^{-6} M very significantly inhibits the start of growth of the primordial follicles as is shown by the maintenance over time of the number of primordial follicles (see Figure 1) and the absence of increase in the number of primary follicles (see Figure 2).

Brief description of the figures

Figure 1 represents the proportions of dormant follicles during a period of 10 days of culture of a ewe's ovarian cortex. These proportions are measured for each sample tested on the day of the start of the experiment (D0), on the 4^{th} day (D4), on the 7^{th} day (D7) and on the 10^{th} day (D10).

Figure 2 represents the proportions of primary follicles during a period of 10 days of culture of a ewe's ovarian cortex. These proportions are measured for each sample tested on the day of the start of the experiment (D0), on the 4^{th} day (D4), on the 7^{th} day (D7) and on the 10^{th} day (D10).

Tests for determination of the agonist or antagonist effect of a somatostatin analogue

Inhibition of the intracellular production of cAMP

CHO-K1 cells expressing the sub-types of human somatostatin (SRIF-14) receptors are cultured in 24-well plates in an RPMI 1640 medium containing 10% foetal calf serum. The medium is changed the day before the experiment.

The cells at a rate of 10^5 cells/well are washed twice with 0.5 ml of new RPMI medium comprising 0.2% BSA completed with 0.5 mM of 3-isobutyl-1-methylxanthine (IBMX) and incubated for approximately 5 minutes at approximately 37° C. The production of cyclic AMP is stimulated by the addition of 1 mM of forskolin (FSK; supplier: Sigma Chemical Co., St. Louis, MO, USA) for 15-30 minutes at approximately 37° C.

Determination of the agonist effect of a somatostatin analogue

The agonist effect of a somatostatin analogue is measured by the simultaneous addition of FSK (1 µM) and the analogue to be tested (10^{-10} M to 10^{-5} M).

The reaction medium is eliminated and 200 ml of HCl 0.1 N are added. The quantity of cAMP is measured by a radioimmunoassay (Kit FlashPlate SMP001A, New England Nuclear, Boston, USA).

Determination of the antagonist effect of a somatostatin analogue

The antagonist effect of a somatostatin analogue is measured by the simultaneous addition of FSK (1 µM) , SRIF-14 (1 to 10 nM) (supplier: Bachem, Torrence, CA, USA) and the analogue to be tested (10^{-10} M to 10^{-5} M).

The reaction medium is eliminated and 200 ml of HCl 0.1 N are added. The quantity of cAMP is measured by a radioimmunoassay (Kit FlashPlate SMP001A, New England Nuclear, Boston, USA).

Claims

1. Use of somatostatin or one of its agonist analogues for preparing a medicament intended to regulate the ovarian follicular reserve, and in particular to reduce the depletion of the ovarian follicular reserve over time, in non-menopausal women.

2. Use according to claim 1, characterized in that somatostatin is used for preparing the medicament.

3. Use according to claim 1, characterized in that a somatostatin agonist analogue is used for preparing of the medicament.

4. Use according to claim 3, characterized in that the somatostatin agonist analogue is chosen from the group comprising lanreotide, octreotide, vapreotide, SOM 230, MK-678, BIM-23190, BIM-23197, BIM-23268, PTR-3173, TT-232 and their pharmaceutically acceptable salts.

5. Use according to claim 4, characterized in that the somatostatin agonist analogue is lanreotide or one of its pharmaceutically acceptable salts.

6. Use according to one of claims 1 to 5, characterized in that the medicament is intended to be administered to a woman at risk of early menopause.

7. Use according to one of claims 1 to 5, characterized in that the medicament is intended to be administered to a woman who has an X chromosome micro-deletion.

8. Use of a somatostatin antagonist analogue for preparing a medicament intended to accelerate the start of growth of the quiescent follicles in non-menopausal women.

9. Use according to claim 8, characterized in that the somatostatin antagonist analogue is chosen from the peptides of general formula **(I)**

$$A^1\text{-cyclo}\{\text{D-Cys-}A^2\text{-D-Trp-}A^3\text{-}A^4\text{-Cys}\}\text{-}A^5\text{-}Y^1$$

(I)

in which:

A^1 is an optionally substituted aromatic α-amino acid;

A^2 is an optionally substituted aromatic α-amino acid;

A^3 is Dab, Dap, Lys or Orn;

A^4 is β-Hydroxyvaline, Ser, Hser, or Thr;

A^5 is an optionally substituted aromatic D- or L- α-amino acid; and

Y^1 is OH, NH_2 or NHR^1, R^1 being (C_{1-6})alkyl;

each optionally substituted aromatic α-amino acid being optionally substituted with one or more substituents independently chosen from the group comprising a halogen atom and the groups NO_2, OH, CN, (C_{1-6})alkyl, (C_{2-6})alkenyl, (C_{2-6})alkynyl, (C_{1-6})alkoxy, Bzl, O-Bzl and NR^9R^{10}, R^9 and R^{10} each being independently H, O, or (C_{1-6}) alkyl; and

each nitrogen atom with a peptide amide bond and the amino group of A^1 being optionally substituted with a methyl group, it being understood that there is at least one such methyl group in a peptide of general formula **(I)**;

and the pharmaceutically acceptable salts of such peptides.

10. Use according to claim 8, characterized in that the somatostatin antagonist analogue is chosen from the group comprising:

- the following peptides:

  Cpa-cyclo[D-Cys-Pal-D-Trp-N-Me-Lys-Thr-Cys]-D-Trp-NH_2;

  Cpa-cyclo[D-Cys-Tyr-D-Trp- N-Me-Lys-Thr-Cys]-Nal-NH_2;

  Cpa-cyclo[D-Cys-Pal-D-Trp- N-Me-Lys-Thr-Cys]-Nal-NH_2;

- the peptide known by the code name AC-178,335;
- the octapeptide known by the code name ODN-8;
- the peptide known by the code name SB-710411;
- the peptide known by the code name BIM-23056;
- the compound known by the code name BN-81674;
- the compound known by the code name SRA-880;

and their pharmaceutically acceptable salts and protected forms.

Abstract

The invention relates to the use of somatostatin or one of its agonist analogues for preparing a medicament intended to regulate the ovarian follicular reserve, and in particular to diminish the depletion of the ovarian reserve through time, in non-menopausal women, or to the use of a somatostatin antagonist analogue for preparing a medicament intended to accelerate the start of growth of the quiescent follicles in non-menopausal women.

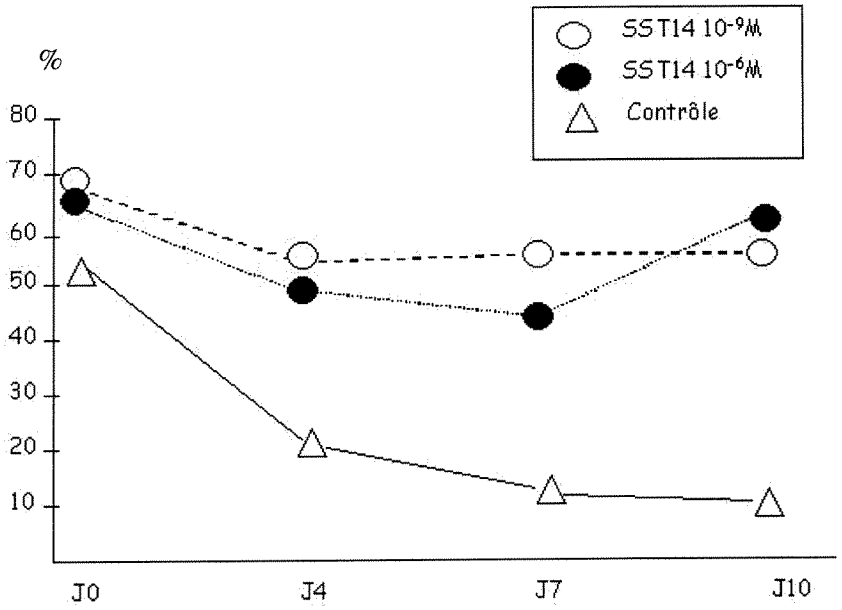


Figure 1

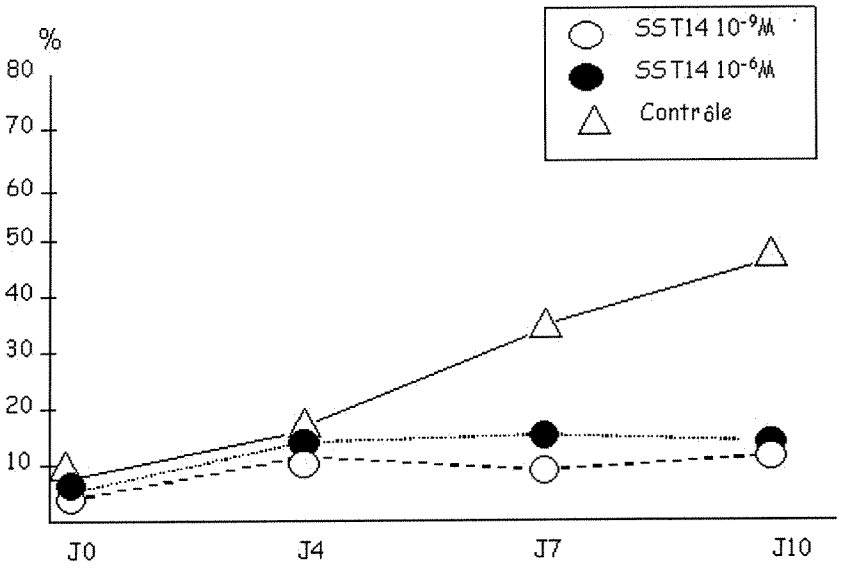


Figure 2